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Center For Reproduction Of Endangered Species

1990-91 Winter Report

Editor: Judy Kinsell

- **GENETICS** CRES geneticist Dr. Oliver Ryder recently traveled to the U.S.S.R. to invite the Soviets' participation in a global management plan for the Przewalski's horse. The Soviets agreed to collaborate in an international breeding program for the horses to help preserve the gene pool. Collaboration in efforts to reintroduce the species to Soviet Central Asia and the Mongolian People's Republic was formalized through discussions with Soviet officials.

While in the Soviet Union, Dr. Ryder visited Askania Nova, a nature reserve in the Ukraine and home to 120 Przewalski's horses—the largest single collection in existence. There, Dr. Ryder reached an agreement with officials to exchange two stallions each with the Zoological Society of San Diego in 1991. He discussed the sanctuary's complete herd management and obtained blood samples for genetic analysis.

Another new approach in working toward gene pool management is to use the resources of museums. In Leningrad Dr. Ryder was able to take skin samples from preserved specimens of Przewalski's horses owned by the Czar. "It was like holding history in your hands," he said. "These samples will tell us a lot about the geographic variations in gene pools and how they existed before populations got to their current depressed levels."

The Przewalski's horse, a native of Mongolia, is the last true species of wild horse. It was first described to the western world in 1881. The discovery of this unique animal resulted in captive breeding efforts, without which this species would surely be extinct. In fact, all Przewalski's horses living today trace their ancestry to 12 animals brought out of Mongolia at the turn of the century, and a single mare captured in 1947.

In tracking the inheritance of remaining Przewalski's horses, the CRES genetics division is looking at mitochondrial DNA in the museum samples from the Soviet Union. In mitochondrial DNA, traits are inherited mother to daughter, so females are referred to as maternal clones. Males are dead ends. "All surviving Przewalski's horses are from only four different maternal lineages," explained Dr. Ryder. "So, we can look at the pedigrees and see how many of the founder females have uninterrupted chains from daughter to daughter to daughter, etc. We have discovered a genetic feature that distinguishes today's Przewalski's horses from domestic horses. A retrospective analysis of the museum samples will give us a broader, more accurate look at the gene pool to see if this same genetic feature can be traced."

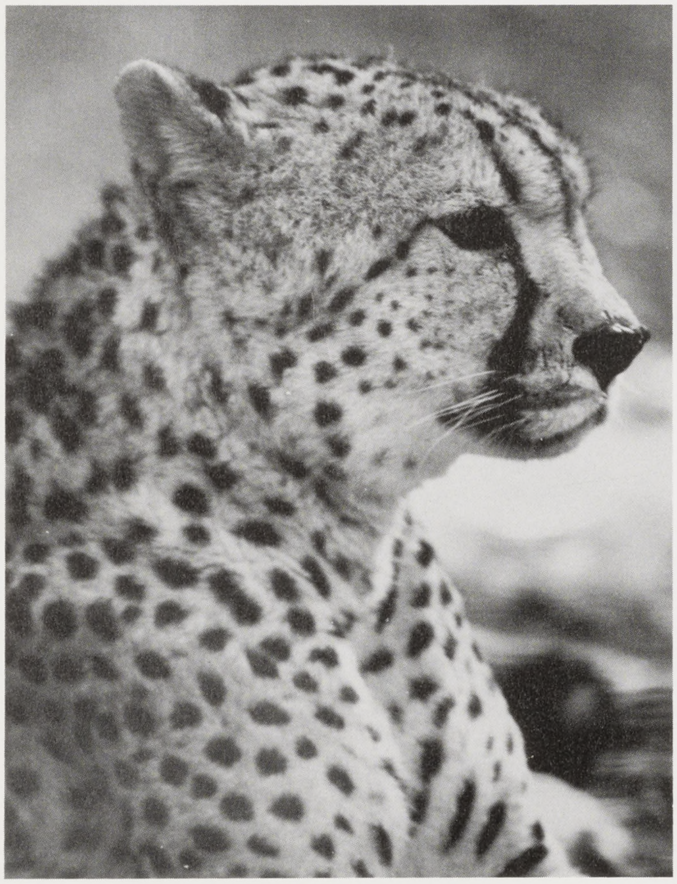
• ENDOCRINOLOGY

Familiarity between male and female cheetahs discourages them from responding to each other as mates. So, at the Wild Animal Park's cheetah breeding facility males and females live separately as they would in the wild. This arrangement poses one obvious problem—how to know when a female is in estrus so she can be paired with a male for breeding.

CRES endocrinology specialist Nancy Czekala-Gruber and behaviorist Dr. Donald Lindburg were awarded a $25,000 matching grant from the Institute of Museum Services to investigate hormones as indicators of estrus and pregnancy in female cheetahs. Determining estrus in cheetahs is particularly difficult because of "silent estrus," in which the animals show no recognizable behavioral changes during the 24-to-48-hour ovulation period.

Previous charting of urinary hormones proved futile, so Ms. Czekala-Gruber's study involved analyzing daily fecal samples of female cheetahs who were neither pregnant nor nursing. "We followed five estrus cycles and monitored three pregnancies. The charted fecal estrogen and progesterone will indicate in future analysis the ovulatory period and pregnancy detection," she said.

"Reproductive physiologist Dr. Barbara Durrant used vaginal cytology (swabbing for cell collection) on one tractable female as a marker in this study. When the activity charted through vaginal cytology correlated with the rise in hormones from the fecal samples, we knew we had found a reliable indicator."



A number of zoos were asked to participate in the cheetah hormone study, and findings were well received. "In the future," said Ms. Czekala-Gruber, "the fecal study may be used to find a behavioral marker that will make it much easier to determine estrus consistently."

• REPRODUCTIVE PHYSIOLOGY

CRES reproductive physiologist Dr. Barbara Durrant and her team are searching for a reliable means of freezing and thawing cheetah semen for artificial insemination. In all but a very few cases, freezing has damaged the sperm and destroyed its fertilizing ability. To discover how cryopreservation of cheetah sperm can be performed with a greater degree of success, Dr. Durrant is investigating the structure of cheetah sperm.

"We're concentrating on looking at the acrosome, which is the part of the sperm that actually makes contact with the membranes of the egg," explained Dr. Durrant. "If the acrosome is not intact, the sperm cannot penetrate the egg because it can't make the appropriate biochemical connections to the egg."

"There's a process called the acrosome reaction that must occur at the time of sperm/egg contact for fertilization to be successful. If we look at the acrosome of frozen sperm, we can get an idea if the sperm have the potential to penetrate the egg. The acrosome can be damaged during freezing, so by assessing the extent of acrosomal damage we can evaluate different freezing protocols."

Cheetah sperm are so small that the acrosomes must be stained to be seen under a light microscope. Dr. Durrant chose a chlorotetrocycline (CTC) stain because tetrocylene fluoresces.

A closer look at the acrosome shows it is composed of three membrane layers. The plasma membrane surrounds the entire head of the sperm. Underneath are the outer acrosomal membrane and inner acrosomal membrane.

"What we're going to do," said Dr. Durrant, "is to sequentially strip those three membranes. Using the electron microscope at the Beckman Laser Institute, we'll take a close look at the sperm to see what we've actually done by stripping those membranes off. As we strip each membrane, we'll use the CTC stain to look at the resulting staining patterns."

The results will indicate the integrity of the acrosome and validate the CTC procedure. Dr. Durrant is hopeful that the CTC acrosome evaluation will provide an effective way to evaluate sperm and to aid in discovering a way to prevent frost damage.

Led by Dr. Werner Heuschele, CRES scientists are in a race against time to save endangered animal species. Their accomplishments are shared with zoos and wildlife organizations worldwide for the benefit of those who join us in our commitment.

For information on how you can help CRES, please call Amy Parrott at (619) 231-1515, extension 4249.

● **CONSERVATION AWARD** Gilbert M. Grosvenor, president of the National Geographic Society, was awarded the Conservation Medal of the Zoological Society of San Diego on October 19. Mr. Grosvenor accepted the prestigious medal at a dinner for supporters of the Zoological Society's Center for the Reproduction of Endangered Species.

As president and chairman of the board of the National Geographic Society, the world's largest nonprofit scientific and educational institution, Gilbert Grosvenor has dedicated his career to pursuing a timeless goal that was first articulated by his great-great-grandfather when he helped to establish the organization more than 100 years ago: to increase and diffuse geographic knowledge.

Mr. Grosvenor joins a list of Conservation Medal holders that includes Jane Goodall, Sir David Attenborough, Prince Phillip, Ian Player, Norman Myers, Russell Mittermeier, and San Diegans Charles Schroeder, Kenton C. Lint, Kenhelm W. Stott, Jr., the late Charles Shaw and the late Carl L. Hubbs.

The Conservation Medal was first awarded in 1966. It is presented by the Zoological Society's board of trustees to individuals who have significantly increased knowledge of wildlife habits and habitats, who have played an active role in wild species preservation, or have furthered the cause of conservation through financial support, influence or publicity.

● **BEHAVIOR** All female monkeys, great apes and humans who continuously breastfeed normally experience a period of post-partum infertility. This occurs when the hormone prolactin supresses the production of estrogens and progestins, which are necessary for a normal menstrual cycle to resume. But why do interbirth intervals vary so dramatically between certain species? CRES research associate Dr. Nancy Harvey recently conducted an investigation to determine why it takes longer for lactating lion-tailed macaques to conceive after giving birth than other species of macaques.

Dr. Harvey's is the first scientific study of its kind. "No one has ever investigated the hormones and behaviors of lactating female lion-tailed macaques to explain this phenomenon," she said. "We had an excellent opportunity to conduct this study, because six lion-tailed macaque babies had been born a few months previously."

When hormones from urine samples of lactating females were analyzed, Dr. Harvey discovered some extremely unusual patterns. "The progestins, which I thought would be non-existent following pregnancy and lactation, were elevated well beyond the levels you would find in a normal reproductive cycle. But the estrogens, which we expected to be abnormal in pattern those first few recovery cycles, were not." Even though the macaques were copulating and the females had swellings, none of them conceived until the progestins began to follow a normal course.

This evidence suggests the extremely high levels of progestins found in the first lactational recovery cycles were in fact a result of hormonal activity from the previous pregnancy. Only after several cycles did the progestins begin to follow a normal course that allowed females to conceive again. Further studies are underway to investigate social and environmental factors that may contribute to the lion-tailed macaques' long interbirth interval.

The Zoological Society of San Diego has 42 lion-tailed macaques in its collection—more than 10 percent of the world's captive population. Our thriving social groups of this endangered species enable CRES researchers to learn more about the breeding and development of these rare animals.

● **COMPARATIVE PHYSIOLOGY** CRES comparative physiologist Dr. John Phillips is presently in Namibia, Africa, studying the reproductive biology of the endangered savannah monitor lizard. Thus far the investigation has yielded valuable information that may lead to successful captive reproduction of this and related species.

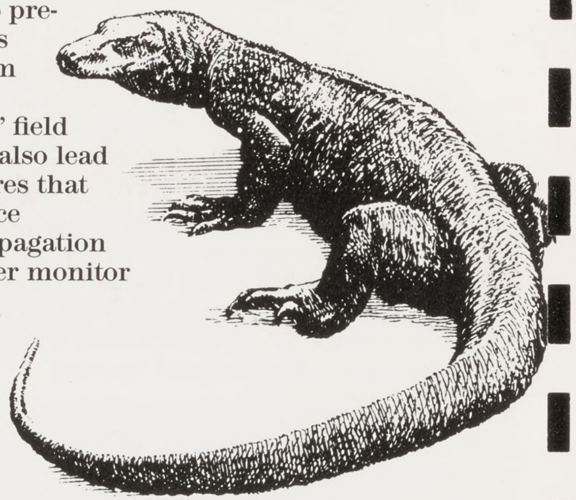
At Etosha National Park, Dr. Phillips followed 30 to 40 animals, but focused on 11 pairs. One or both members of each pair were radio collared, and their whereabouts tracked electronically. Every three weeks, blood samples were taken to measure the females' hormone levels.

"One interesting thing we found," said Dr. Phillips, "is that in the wild, males and females are never together except during mating season. But in captivity, pairs are constantly together. This may explain why captive monitors have not reproduced successfully."

In the wild, all female savannah monitors go into estrus once a year at mating season, which occurs toward the end of August. "What was surprising," said Dr. Phillips, "is that their estrogen levels go up so dramatically—a 30,000 percent increase over the baseline level. We've never seen estrogen levels anywhere near as high in captive monitors as in the wild. This suggests our captive females have never cycled."

When Dr. Phillips returns to San Diego in July he plans to bring with him between 25 and 35 savannah monitor hatchlings to begin a breeding program at the Wild Animal Park. Applying information gained from the field study, males and females will be housed separately and given plenty of space—about 100 square feet of territory per lizard.

Understanding why the reproductive cycle shuts down in the captive female monitors will bring us closer to preserving this species from extinction. Dr. Phillips' field study may also lead to procedures that will enhance captive propagation among other monitor lizards, including the giant komodo dragon.



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Zoological Society Of San Diego
Development Department
Post Office Box 551
San Diego, California 92112

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